Original Research Article

Effect of Rhus tox on Micropropagation of *Scoparia dulcis* L. through leaf explants culture

ABSTRACT

Scoparia dulcis L. is an erect annual herb that shows hypoglycemic, anti-tumour promoting, anti-viral activities, analgesic and antipyretic activities. The major phytochemicals of this plant include coumarins, phenols, saponins, tannins, amino acids, flavonoids, terpenoids, catecholamines, noradrenaline and adrenaline. The active principles of *Scoparia dulcis* include scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin.

The present study deals with the micropropagation of this plant through leaf explants culture in the MS medium supplemented with different concentration of IAA, IBA, NAA, BAP and Kinetin, and Rhus tox 30, a homeopathic medicine. The results indicated that the IAA in the concentration range of 0.10 to 2.0 mg/l and BAP in the concentration range of 0.10 to 2.5 mg/l induced callus formation from the leaf explants. IAA in the concentration range of 0.10-0.30 mg/l and BAP in the concentration range of 0.10-0.30 mg/l induced green, compact type of calli. However, IAA in the concentration range of 0.10 to 0.30 and 0.50 mg/l BAP induced the formation of brown friable calli. IAA in the concentration of 0.50 mg/l and 0.10-0.50 mg/l of BAP induced the formation of brown, friable calli. IAA/BAP concentration of 1.50-2.0/1.50-2.5 mg/l induced the formation of green, compact calli. 2, 4-D/Kinetin at 1.5 mg/l/1.5-6.5mg/l concentrations induced only brown, friable calli. The Rhus tox 30 at concentration of 100 µl/l caused enhanced proliferation of leaf explants. At 2,4-D/Kinetin concentration of 1.5/1.5mg/l plus Rhus tox 30, the weight of callus was 520.0±1.12 mg after 42 days of incubation. The weight of callus increased to 1092±0.74 mg after 42 days of incubation with 2,4-D/Kinetin in concentration of 1.5/5.5 mg/l plus Rhus tox 30 (100µl/l). The plantlets were hardened and established with more than 88% success rate.

The development of micropropagation protocol will facilitate access to natural and induced variations in near future. In addition to plant growth regulators, Rhus tox 30 might be useful in enhanced callus formation and plantlet regeneration.

Key Words: *Rhus tox 30, Scoparia dulcis, Micropropagation, Leaf explants, IAA, IBA, α-NAA, BAP, Kinetin*

1. INTRODUCTION

Scoparia dulcis L. commonly known as Broomweed or Sweet broom belongs to family Scrophulariacea of Dicotyledonous angiosperm. It is an erect annual herb, measuring about half meter in height and distributed in tropical and subtropical regions. The plant bears serrated leaves and white flowers. Flowers are complete, bisexual, axillary, tetra fid, rotate and measure about 6-7 mm in diameter. Sepals 5, gamosepalous with oval-oblong calyx lobes, three nerved, glabrous, ciliated at the margin and persistent. Petals 5, gamopatelous, pale yellow to white; corolla tube densely hairy at the throat; aestivation imbricate; corona present; corolla lobes 2-4 mm long, apex of petals obtuse, slightly curved, upper lobes slightly larger than others. Stamens 4 exerted; filament inserted at the top of the corolla tube; anthers dorsifixed. Carpels 2, syncarpous, ovary superior, unilocular; ovules anatropous,

numerous on axile placentation; style erect, about 2 mm long; stigma truncate to bipartite. Flowering time is throughout the year.

The ethnomedicinal uses of *Scoparia dulcis* are well documented [1] and show hypoglycemic, anti tumour promoting, anti-viral activities, analgesic and antipyretic activities [2-8]. *Scoparia dulcis* is rich in flavonoids and terpenes and pharmacological properties of this plant is due to the presence of these phytochemicals [9].

The major phytochemicals of this plant include coumarins, phenols, saponins, tannins, amino acids, flavonoids, terpenoids and catecholamines [10]. Noradrenaline and adrenaline are also present in addition to these phytochemicals [11] which possess sympathomimectic effects. The active principles of *Scoparia dulcis* include scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin [12, 13]. These active principles possess inhibitory activity against replication of herpes simplex virus (HSV), gastric H+, K+ ATPase activator and antitumor promoting activity [12]. The terpenoids viz. alpha-amyrin, betulinic acid, dulcioic acid, friedelin, glutinol and ifflaionic acid have also been identified [14-16].

Pharmacological activities of *Scoparia dulcis* have been investigated and known to possess antidiabetic actiovity [17, 18, 19] (Pari et al., 2004; Latha and Pari, 2004; 2005), analgesic and antitumour activity, antiviral activity [20-22] (Hayashi *et al*., 1988, 1990, 1992), antimalarial activity [23] (Riel et al., 2002), neurotrophic activity [24, 25] (Li and Ohizum, 2004; Li et al., 2004), anticancer activity [26, 27] (Nkembo et al., 2005; Nakagiri et al., 2005), diuretic activity [28, 19] (Ahmed et al., 2001; Freire et al., 1991).

Plant tissue culture is being used for the improvement and rapid multiplication of medicinal plants [29, 30]. *Scoparia dulcis* is recognized as medicinal plant and the present study deals with the micropropagation of this plant through leaf explants culture in the MS medium supplemented with 100 µ/ml of Rhus tox 30, a homeopathic medicine obtained from a plant *Rhus toxicodendron* Linn. (Poison ivy) of family Anacardiaceae. Rhus tox is used in the treatment of cramps, strains, sprains, restless leg syndrome, flu, viral infections and arthritis.

2. MATERIALS AND METHODS

2.1. Place and Duration of Study

The present work was carried out in Plant Tissue Culture Laboratory, Department of Botany, Patna Science College (Patna University) Patna (India) between September 2018 and August 2019.

2.2 Plant Collection, Identification and Sterilization

The healthy plants of *Scoparia dulcis* (Scrophulariaceae) grown in the herbal garden of Department of Botany, Patna Science College, Patna were collected and identified following standard monographs and used as experimental material. The fresh leaves were excised from the twigs and washed under running tap water for twenty minutes. The leaves were cut into 2-3 cm pieces. The explants were first washed with 5% savlon solution (v/v) and then with distilled water 4 to 5 times. The surface sterilization was carried out first by immerging in 70% ethanol (v/v) for 45 seconds and then by immerging in 0.1% (w/v) mercuric chloride solution for five minutes and rinsed with sterilized double distilled water in the laminar air flow.

2.3 Explant Sterilization and Incubation

The explants were dried on sterilized filter paper and inoculated in culture tubes (150 X 25 mm) aseptically containing 15-20 ml of solid Murashige and Skoog's medium (MS medium) [31] supplemented with 3% (w/v) sucrose (PCTO607 HIMEDIA), $CaCl_2$, vitamins and 0.8% (w/v) agar (PCT 0901 HIMEDIA), and varied concentrations of auxins viz. Indole 3-acetic acid (IAA), α-naphthalene acetic acid (NAA) and 2,4-dichloro phenoxy

acetic acid (2,4-D) and cytokinin viz 6-Benzylamino purine (BAP) and kinetin in combination with 100µl of Rhus tox 30. pH was adjusted to 5.6-5.8 and the media were sterilized in autoclave at a pressure of 15 lb/square inch and temperature of 121^0C for 15 minutes. The cultures were maintained in the culture room at a temperature of 25 ± 2^0C and relative humidity (RH) of 60-70% at a light intensity of 40-50 $\mu molm^{-2}s^{-1}$ under a photo period of 16/8 hr (light/dark). A minimum of 15 cultures were raised and the experiments were conducted in replicates of three. The cultures were maintained regularly by sub culturing at monthly intervals. The number of explants producing calli was recorded after four weeks of culture. The calli were transferred to fresh media supplemented with BAP, 2, 4-D and Rhus tox for initiation of shoots. The number of shoots produced per callus was recorded in every week. When shoots became 2-3 cm in length they were excised transferred to rooting media vertically in culture tubes containing 15-20 ml of MS medium with different concentrations of Indole 3-acetic acid (IAA) or Indole 3-butyric acid (IBA) or α-napthalene acetic acid (NAA). For each treatment, 25 tubes were inoculated. After 30 days of initial culture, data with respect to cultures producing roots, number of roots per shoots and root length (cm) were recorded. The MS media not supplemented with Rhus tox were considered as control. Rooted plantlets were cleaned to remove agar and transferred to sterile earthen pots containing sand and vermiculite (1:1) and covered with polybags with holes. After two weeks plants were acclimatized in culture room without plastic bags for 5-6 hours. One week after acclimatization plants were transferred to pots containing soil under natural environment for hardening.

2.4 Statistical Analysis

The data for number and length of shoots per explants, and number and length of roots per shoot were statistically analyzed by one way analysis of variance (ANOVA) and significant difference was calculated using Duncan's multiple range tests.

3. RESULTS

3.1. Callus formation

The leaf explants of *Scoparia dulcis* were inoculated in MS medium supplemented with IAA/BAP in the concentration range of 0.1-2.0 mg/l/0.1-1.0 mg/l (Table-1).

Table-1: Effect of different concentrations of IAA and BAP in callus induction from leaf explants of *Scoparia dulcis*

Concentration of Phytohormones in mg/l		Callus types
IAA	BAP	
0.10	1.0	Green, compact
	0.30	Green, compact
	0.50	Brown, friable
0.20	0.20	Green, compact
0.30	0.10	Green, compact
	0.30	Green, compact
	0.50	Green, friable
0.50	0.10	Brown, friable
	0.30	Brown, friable
	0.50	Brown, friable
1.50	1.50	Green, compact
	2.0	Green, compact
	2.5	Green, compact
2.0	1.50	Green, compact
	2.0	Green, friable
	2.5	Green, compact

It was observed that the IAA in the concentration range of 0.10 to 2.0 mg/l and BAP in the concentration range of 0.10 to 2.5 mg/l induced callus formation from the leaf explants. IAA in the concentration range of 0.10-0.30 mg/l and BAP in the concentration range of 0.10-0.30 mg/l induced green, compact type of calli. However, IAA in the concentration range of 0.10 to 0.30 and 0.50 mg/l BAP induced the formation of brown friable calli. IAA in the concentration of 0.50 mg/l and 0.10-0.50 mg/l of BAP induced the formation of brown, friable calli. IAA/BAP concentration of 1.50-2.0/1.50-2.5 mg/l induced the formation of green, compact calli. However, equal concentration of IAA and BAP i.e. 2.0 mg/l each induced the formation of brown friable calli (Table-1; Fig: 1a-g).

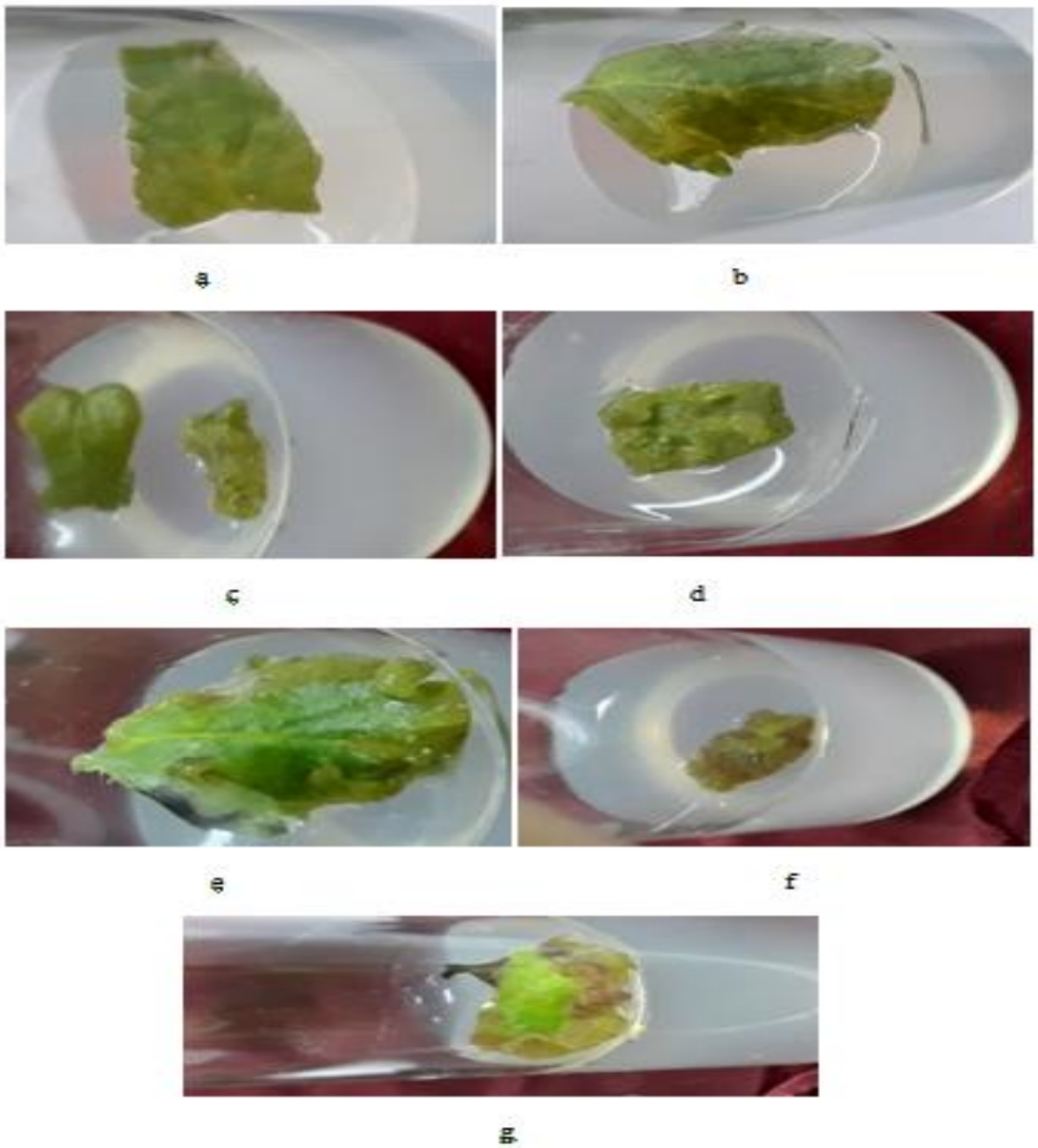

Fig-1a: Induction and growth of callus from leaf explants of *S. dulcis*
Fig-1b: Induction and growth of callus from leaf explants of *S. dulcis*
Fig-1c: Development of green, compact callus
Fig-1d: Development of green, compact callus
Fig-1e: Development of green, friable callus
Fig-1f: Development of brown, friable callus
Fig-1g: Development of brown, friable callus

The effect of 2, 4-D and Kinetin on callus formation was also studied and the results obtained have been presented in Table-2

Table-2: Effect of 2, 4-D and Kinetin on callus formation from leaf explants of *Scoparia dulcis*

Concentration of 2,4-D in mg/l	Concentration of Kinetin in mg/l	Callus types	Days of callus induction
1.5	1.5	Brown, friable	12
1.5	2.5	Brown, friable	10
1.5	3.5	Brown, friable	9
1.5	4.5	Brown, friable	8
1.5	5.5	Brown, friable	8
1.5	6.5	Brown, friable	7

From the result it was observed that when the concentration of 2, 4-D was kept constant i.e. 1.5 mg/l only brown, friable calli were formed at all the concentrations of Kinetin selected in present investigation i.e. from 1.5 mg/l to 6.5 mg/l. However, days of callus induction decreased on increasing the concentration of kinetin. When 2, 4-D and Kinetin concentration were in equal amount i.e. 1.5 mg/l the calli were formed only after 12 days of explants inoculation. The concentration of 2, 4-D/Kinetin in ratio 1.5/2.5 mg/l induced the formation of calli in 10 days; in ratio 1.5/3.5 mg/l in 9 days; in ratio 1.5/4.5-5.5 mg/l in 8 days and in ratio 1.5/6.5 mg/l in 7 days only (Table-2; Fig-2a-g).

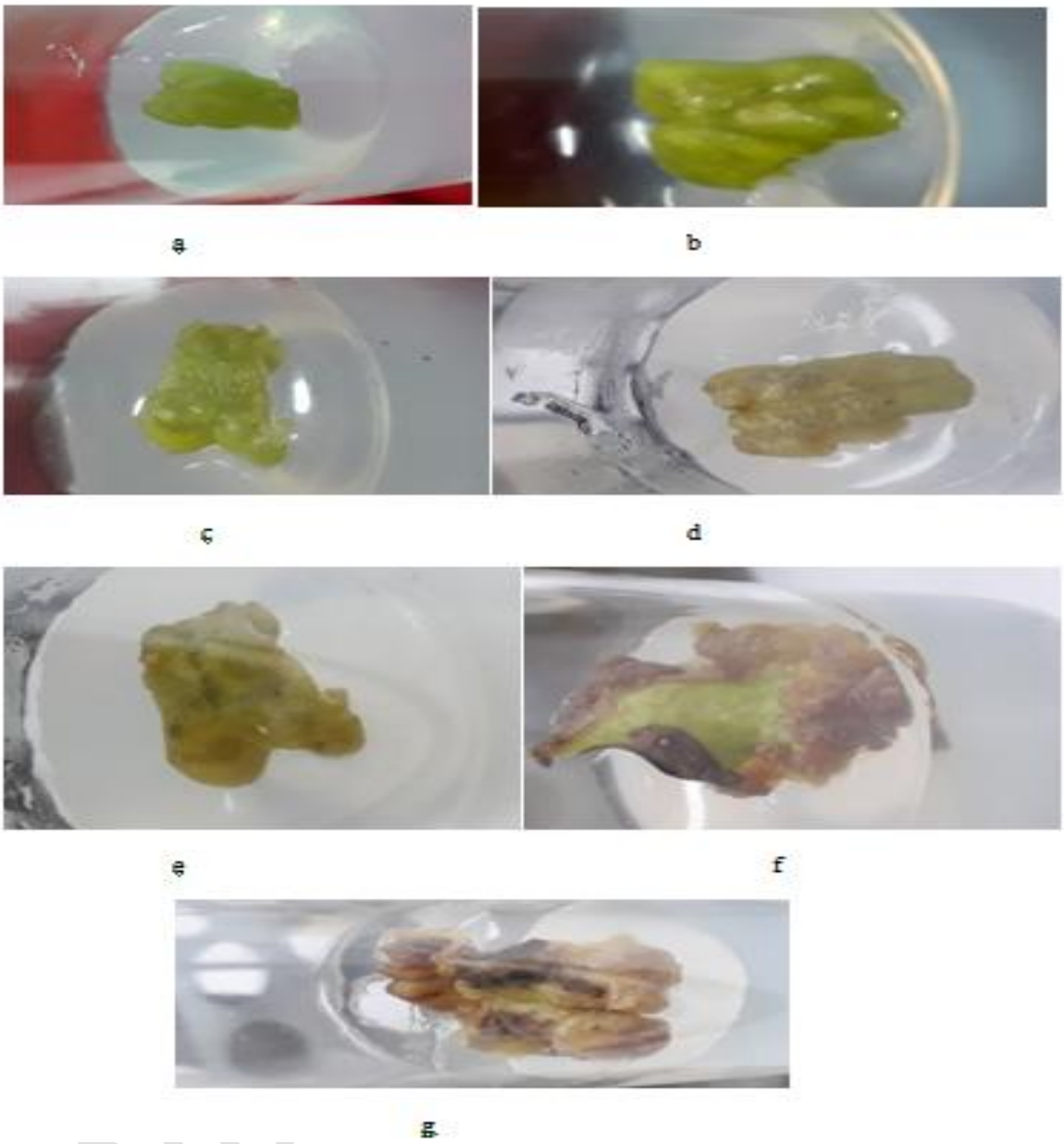

Fig-2: Different stages of callus development in 2, 4-D/Kinetin supplemented medium
Fig-2a: Induction of callus from leaf explants
Fig-2b: Induction of callus from leaf explants after 7 days of inoculation
Fig-2c: Induction of callus development from leaf explants after 8 days of inoculation
Fig-2d: Formation of brown friable calli after 12 days of inoculation
Fig-2e: Formation of brown, friable calli after 9 days of inoculation
Fig-2f: Formation of brown, friable calli after 8 days of inoculation
Fig-2g: Formation of brown, friable calli after 7 days of inoculation

The effect of Rhus tox 30 on callus formation from leaf explants on MS media supplemented with various concentrations of 2, 4-D and Kinetin (from 1.5 mg/l to 6.5 mg/l) was studied. MS medium not supplemented with Rhus tox was treated as control. The results obtained have been presented in Table-3 and 4.

Table-3: Effect of different concentration of Kinetin on callus induction from leaf explants of *Scoparia dulcis*

Concentration of 2,4-D/Kinetin in mg/l	Weight of Callus in mg				Response of culture tubes after inoculation out of ten	Minimum number of days for callus induction	Nature of callus
	Days of incubation						
	10	14	28	42			
1.5/1.5	12.0±0.65	50.6±0.45	127.2±0.54	225.0±1.15	All	10	Pale yellow and soft
1.5/2.5	12.9±0.62	52.1±0.75	350.0±0.63	601.1±1.13	9 out of ten	9	Pale yellow to brown soft friable
1.5/3.5	13.1±0.61	51.3±0.47	400.8±0.64	856.8±0.76	All	8	Brown soft friable
1.5/4.5	13.5±0.47	59.2±0.56	425.2±0.81	901.3±0.66	All	8	Brown soft friable
1.5/5.5	13.4±0.35	60.5±0.27	411.4±0.65	889.2±0.71	All	8	Brown soft friable
1.5/6.5	13.5±0.46	55.0±0.48	398.0±0.35	880.0±0.63	All	8	Brown soft friable

Mean ± SE of 25 replicates; results significant at $P < 0.05$

Table-4: Effect of different concentration of Kinetin and Rhus tox 30 on callus induction from leaf explants of *Scoparia dulcis*

Concentration of 2,4-D/Kinetin in mg/l	Weight of Callus in mg				Response of culture tubes after inoculation out of ten	Minimum number of days for callus induction	Nature of callus
	Days of incubation						
	10	14	28	42			
1.5/1.5	12.0±0.61	55.4±0.43	150.7±0.44	520.0±1.12	All	8	Brown soft friable
1.5/2.5	12.9±0.64	60.3±0.65	400.1±0.61	658.5±1.16	All	8	Brown soft friable
1.5/3.5	13.1±0.61	55.9±0.43	425.4±0.62	900.3±0.71	All	8	Brown soft friable
1.5/4.5	13.5±0.47	69.5±0.51	403.3±0.71	950.6±0.65	All	8	Brown soft friable
1.5/5.5	13.4±0.35	70.0±0.37	895.4±0.65	1092±0.74	All	8	Brown soft friable
1.5/6.5	13.5±0.46	63.1±0.68	385.0±0.55	700.3±0.61	All	8	Brown soft friable

Mean ± SE of 25 replicates; results significant at $P < 0.05$

From the results it is evident that the Kinetin induced enhanced differentiation of leaf explants cells that caused production of calli in highest amount in comparison to control. A concentration of 2, 4-D/Kinetin in equal amount i.e. 1.5/1.5 mg/l caused production of callus after 10 days of incubation. The weight of calli at this concentration was 12.0±0.65 mg. The weight of calli increased on increasing the days of incubation. After 42 days of incubation the weight of callus was 225.0±1.15 mg. All culture tubes produced the pale yellow and soft calli. The kinetin concentration also influenced enhanced callus production. When 2,4-D/Kinetin concentration was 1.5/2.5mg/l only nine culture tubes out of ten exhibited calli formation from leaf explants. At this concentration the weight of callus was 601.1±1.13 mg after 42 days of incubation. At concentration of 1.5 mg/l 2,4-D and 3.5-6.5 mg/l Kinetin only eight culture tubes out of ten were responded for callus formation. The calli produced were brown, soft and friable. The maximum weight of callus was obtained at concentration of 2,4-D/Kinetin in ratio 1.5/4.5 mg/l which was 901.3±0.66 mg after 42 days of incubation. Kinetin concentration of more than 4.5 mg/l caused decline in the weight of callus. At concentration of 6.5 mg/l the Kinetin caused reduction in the weight of callus to 880.0±0.63 mg (Table-3).

The homeopathic medicine Rhus tox 30 at concentration of 100µl/l caused enhanced proliferation of leaf explants. All culture tubes responded equally and produced calli only after eight days of inoculation. The calli were brown, soft and friable. At concentration of 2,4-D/Kinetin of 1.5/1.5mg/l plus Rhus tox 30, the weight of callus was 520.0±1.12 mg after 42 days of incubation. The weight of callus increased to 1092±0.74 mg after 42 days of incubation when MS media were inoculated with 2,4-D/Kinetin in concentration of 1.5/5.5 mg/l plus Rhus tox 30 (100µl/l).

3.2. Shoot induction

The calli derived from leaf explants were used for shoot induction on MS basal media supplemented with variable concentrations IAA and BAP and the results obtained have been presented in Table-5 and Fig-3a-e.

Table-5: Effect of different concentrations of IAA and BAP on shoot production from callus of *Scoparia dulcis*

Concentration of IAA in mg/l	Concentration of BAP in mg/l	Number of cultures producing shoots*	Percent shoot formation	Number of Shoots per callus (Ẍ±SE)	Height of Shoots in cm (Ẍ±SE)
1.0	1.0	21	84	2.45±0.15	7.85±0.25
1.5	1.5	25	100	3.75±0.12	8.65±0.21
2.0	1.5	20	80	1.85±0.16	5.16±0.21
2.5	2.0	16	64	1.55±0.13	4.15±0.13
2.5	2.5	17	68	1.57±0.11	4.17±0.12

Data are Mean ± SE of 25 replicates; * Significant at $P = 0.05$

From the results it is evident that the shoot initiation occurred from callus of leaf explants after 7-8 days of transfer to MS media supplemented with variable concentration of IAA and BAP (Table-5). The most effective concentration of IAA and BAP was 1.5 mg each. At this concentration hundred percent shooting was observed in cultures grown after 28 days. The number of shoots and their length were maximum, 3.75±0.12 and 8.65±0.21 respectively with equal amount of IAA and BAP (1.5 mg/l). The percent shoot formation and the length of shoots decreased with increase in concentration of IAA and BAP above 1.5 mg/l. At 2.5 mg/l concentration of IAA and 2.0

mg/l of BAP the shooting was only of 64 %. The number of shoots and their length were also minimum, 1.55±0.13 and 4.15±0.13 cm respectively (Table-5; Fig-3a-e).

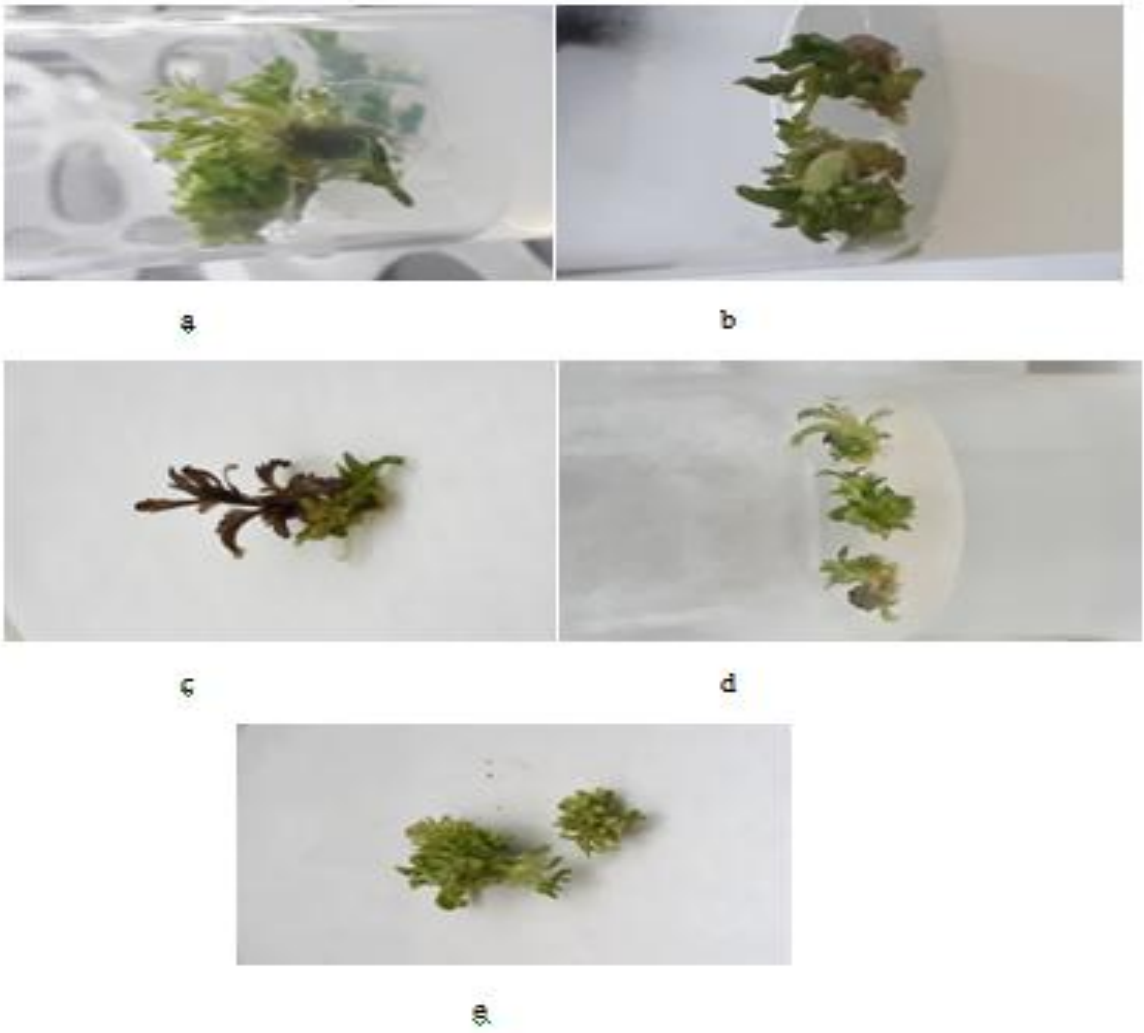

Fig-3a-e: Different stages of shoot formation in MS media supplemented with different concentrations of IAA and IBA

3.3. Root induction

For root induction shoots of about 3 cm in length were transferred to rooting media containing variable concentrations IBA and IAA separately. For this purpose ½ strength of liquid media and solid MS media were used. Filter Paper Bridge was used in case of liquid media. The results obtained have been presented in Table-6-9; Fig 4a-e.

Table-6: Effect of different concentrations of IAA on root production in ½ strength liquid MS media from shoots of *Scoparia dulcis*

Concentration of IAA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per shoot (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	7	21	84	4.45±0.15	3.85±0.35
2.5	9	22	88	4.75±0.12	4.25±0.37
3.5	8	23	92	5.85±0.11	4.36±0.21
4.5	7	25	100	7.45±0.16	6.14±0.26
5.5	7	18	72	3.57±0.75	3.75±0.34
6.5	6	17	68	2.51±0.21	2.71±0.21

Data are Mean ± SE of 25 replicates; * Significant at $P = 0.05$

Table-7: Effect of different concentrations of IAA on root production in solid MS media from shoots of *Scoparia dulcis*

Concentration of IAA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per shoot (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	7	21	84	2.45±0.13	2.87±0.29
2.5	9	21	84	3.75±0.11	3.65±0.21
3.5	8	25	100	5.25±0.16	6.25±0.23
4.5	7	23	92	4.56±0.14	4.25±0.15
5.5	7	20	80	3.57±0.13	3.97±0.16
6.5	6	17	68	2.81±0.17	2.25±0.21

Data are Mean ± SE of 25 replicates; * Significant at $P = 0.05$

Table-8: Effect of different concentrations of IBA on root production in ½ strength of liquid MS media from shoots of *Scoparia dulcis*

Concentration of IBA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per callus (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	8	21	84	3.65±0.25	3.85±0.26
2.5	8	22	88	3.75±0.22	4.65±0.21
3.5	7	23	92	4.85±0.36	5.16±0.22
4.5	7	25	100	5.65±0.17	6.15±0.23
5.5	7	18	72	3.47±0.18	4.17±0.17
6.5	6	17	68	2.65±0.16	3.75±0.15

Data are Mean ± SE of 25 replicates; * Significant at $P = 0.05$

Table-9: Effect of different concentrations of IBA on root production in solid MS media from shoots of *Scoparia dulcis*

Concentration of IBA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per callus (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	8	21	84	2.35±0.17	2.75±0.25
2.5	8	21	84	3.65±0.16	2.85±0.25
3.0	7	25	100	5.85±0.12	5.65±0.24
4.5	7	20	80	4.55±0.17	4.25±0.23
5.5	7	17	68	3.57±0.19	4.13±0.16
6.5	6	15	60	2.75±0.21	3.15±0.11

Data are Mean ± SE of 25 replicates; * Significant at $P = 0.05$

From the results (Table-6) it is evident that the number of shoot let culture producing roots, percent root formation, number of roots per shoot let and their length increased with increase in concentration of IAA in ½ strength liquid MS media. At 1.5 mg/l IAA the root formation started in 7 days of incubation. The number of shooted culture producing roots was 21 out of 25. The percent root formation, number of roots per shoot and their length were 84%, 4.45±0.15 and 3.85±0.35 cm respectively. These values increased on increasing the concentration of IAA up to 4.5 mg/l. At this concentration the rooting started in 7 days of inoculation. All the shoots produced roots i.e. 100% rooting occurred. The number of roots per shoot and their length was 7.45±0.16 and 6.14±0.26 respectively. The percent rooting, number and their length decreased greatly on increasing the concentration of IAA beyond 4.5 mg/l. In solid MS media a more or less similar results was observed. However, the number of roots produced and their length was comparatively less than the ½ strength liquid media. In solid MS media 100% rooting occurred at 3.5 mg/l of IAA. The number of roots per shoot and their length was 5.25±0.16 and 6.25±0.25 cm respectively (Table-7). A more or less similar result of root induction was noticed in ½ strength liquid MS media and solid MS media supplemented with IBA (Table-8 and 9).

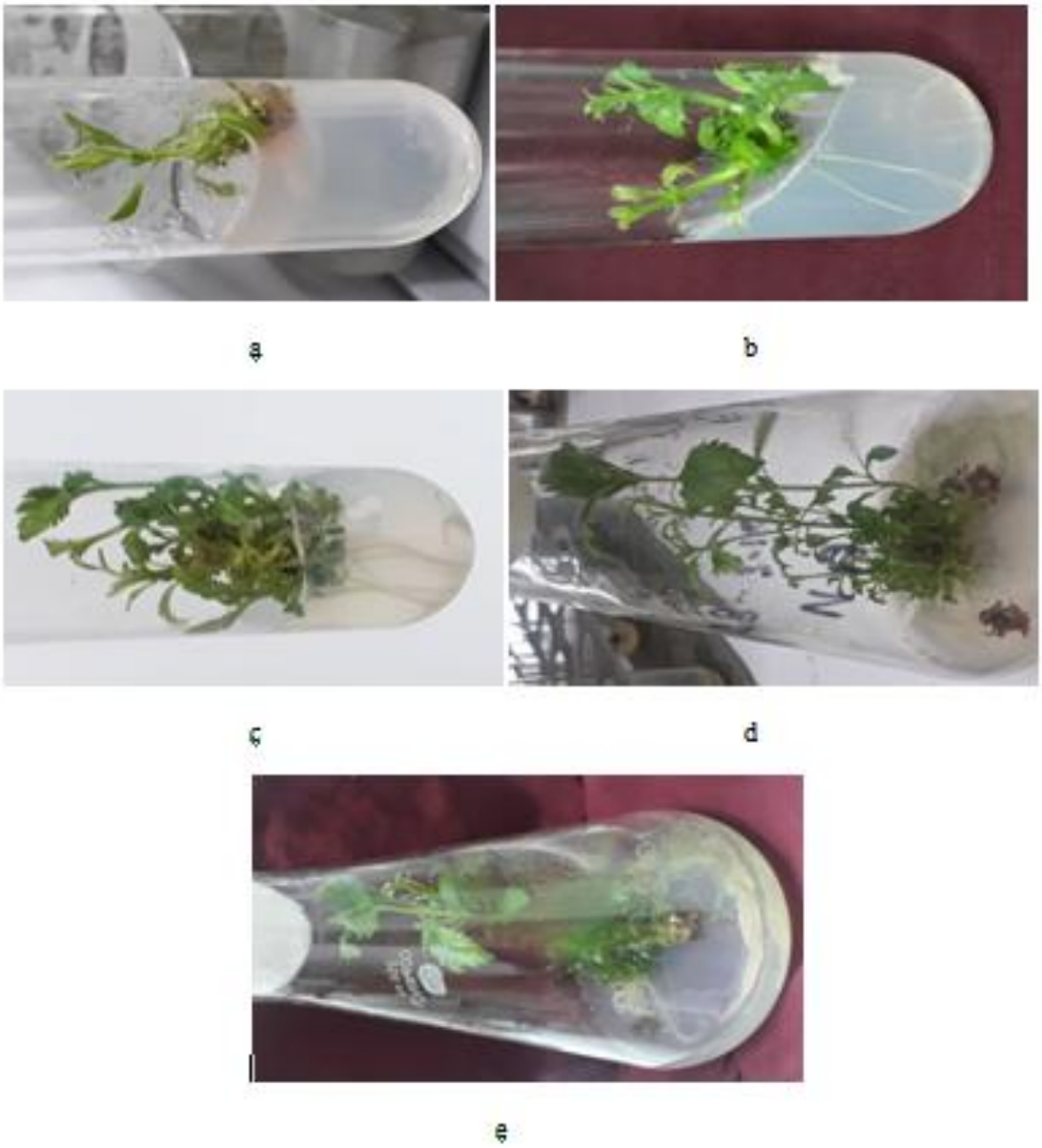

Fig-4a-e: Different stages of root induction in MS media supplemented with IAA and IBA

3.4. Acclimatization of Plantlets

In vitro developed plantlets of about 10-12 cm were subjected to a hardening schedule. The gradual elimination of sucrose and plant growth regulators supported the growth of a well balanced shoot and a well balanced root system. The rooted plantlets were transferred to earthen pots containing sand and vermiculite (1:1). The pots were covered with plastic bags and kept in laboratory at 25 ± 2^{0}C. After 25 days the plants were transferred to soil under natural environment. The survival rate was found to be as much as 88% (Fig-5a-d).

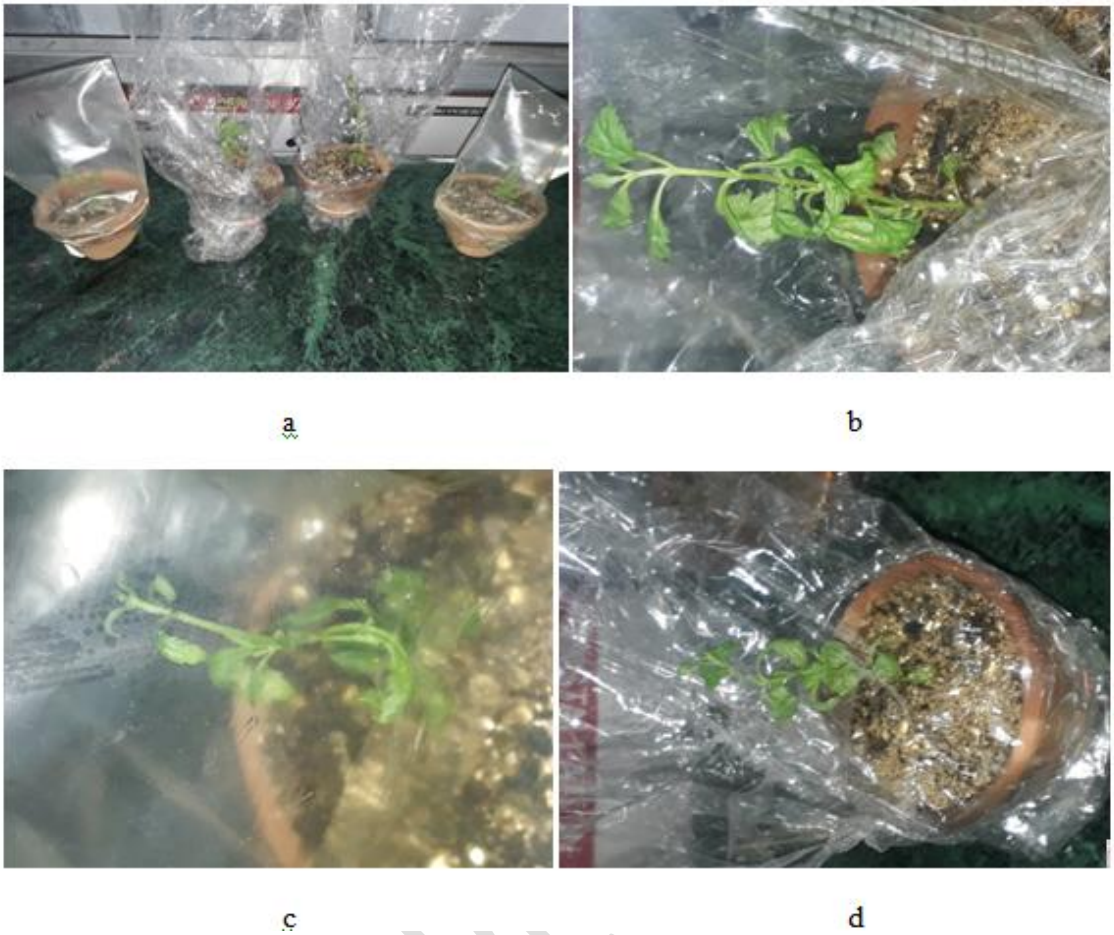

Fig-5a-d: Different stages of acclimatization processes

4. DISCUSSION

The plant growth regulators viz. auxins (IAA, IBA and NAA) and cytokinins (BAP and Kinetin) were considered more important for micropropagation through leaf explants in some other plants also [32, 33]. The BAP in combination with IAA or Kinetin in combination with IBA was found suitable for shoot differentiation from callus developed from leaf explants of *Scoparia dulcis*. The present findings gain support from the work of Sakthi and Mohan [34] who studied the micropropagation of *Scoparia dulcis* from their leaf explants and nodal segments and found more or less similar results. A more or less similar result was also noticed by Kothari and Chandra [35-38], Benavides and Caso [39], Gita Rani *et al*., [40] in case of leaf callus and nodal segments culture of *Tagetes erecta* L.

5. CONCLUSIONS

Micropropagation of *Scoparia dulcis* was carried out on MS medium supplemented with auxins viz. 2, 4-dichloro phenoxy acetic acid (2, 4-D), Indole acetic acid (IAA), Indole butyric acid (IBA) and Cytokinins viz. 6-benzylamino purine (BAP and Kinetin). The homeopathic medicine Rhus tox induced enhanced formation of callus from leaf explants. The maximum numbers of shoots were produced in the combination of BAP and IAA/ Kinetin and IBA. Transfer of shoots (about 3 cm) to MS solid and and ½ strength liquid MS medium favoured rooting in about 28 days and rooted plants (about10cm) were hardened and established with more than 88% success rate. It can be concluded that the development of micropropagation protocol for medicinally important plant species will facilitate

access to natural and induced variations in near future. In addition to plant growth regulators, Rhus tox 30 might be useful in callus formation. The bioactive principles responsible for plant growth promontory activity of Rhus tox 30 obtained from *Rhus toxicodendron* L. demands further investigation at scientific level.

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